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Review

DNA vaccines against cytomegalovirus: current progress

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Abstract

The development of a vaccine for the prevention of primary cytomegalovirus (CMV) infection is a major public health priority. Live attenuated virus, recombinant viral vector, recombinant protein and peptide vaccines have been studied as potential vaccine candidates. In recent years, DNA vaccination strategies have been developed for many pathogens, including CMV. This review aims to bring together many aspects of this relatively new vaccine technology as applied to current research into the development of vaccines against CMV. © 2002 Elsevier Science B.V. and International Society of Chemotherapy. All rights reserved.

Keywords: DNA vaccines; Cytomegalovirus; Antibody; CTL; Protection

1. Introduction

The β-herpesvirus, cytomegalovirus (CMV) infects the majority of individuals during their lifetime yet results in disease only in those whose immune system is immature or impaired by immunosuppressive drugs or human immunodeficiency virus. This virus infects 0.3–2.4% of neonates born in different countries making it the most important cause of intrauterine infection [1]. There is an estimated 1% chance of developing primary CMV infection whilst pregnant in women who enter pregnancy seronegative for CMV [2,3].

The development of a CMV vaccine for the prevention of primary CMV infection is thus a major public health priority. A current report from the Institute of Medicine strongly supports the development of a CMV vaccine based on the economic impact of the disease caused by this virus [4]. A study published recently by our group using a mathematical modelling approach calculated that the critical vaccination proportion required for eradication of CMV in the developed world lies between 59 and 62% demonstrating that even if a putative vaccine were only 80–90% effective in preventing primary infection, CMV could be eradicated from the population by the immunisation of 66–75% of the population, a target easily achievable given a 90% current routine paediatric immunisation rate [5].

Of the 200 genes encoded by the CMV genome [6], only a small proportion are thought to comprise the targets of immune responses necessary for protection against CMV [7]. The neutralizing antibody response against HCMV is predominantly directed against a single protein, glycoprotein B (gpUL55) [8,9] while the tegument protein pp65 (ppUL83) is a major target of the cellular immune response [10–12]. These antigens have formed the basis of the majority of vaccine candidates thus far, which encompass their use within recombinant viral vectors [13,14], recombinant protein vaccines [15] and peptide vaccines [16]. Other candidate vaccine antigens have been proposed or studied for inclusion into a CMV vaccine. HCMV gH is a major target for complement-independent neutralizing antibody response in animals and humans and is thus a candidate antigen [17]. gH requires the assistance of another protein, gL for transport to the cell surface [18] which may thus need to be included in the vaccine preparation. It has recently been shown that 62% of sera from HCMV-seropositive donors reacted with the HCMV gM–gN complex, which thus may represent a major antigenic target and vaccine candidate [19,20]. With regards the generation of cellular responses, the non-structural IE1-exon4 protein has been shown to be an important CTL target [21].

Over the last few years, DNA vaccines have been shown to be effective inducers of cellular and humoral responses against viral antigens [22,23]. This review aims to bring together many aspects of this new vaccine

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technology as applied to current research into the development of vaccines against CMV.

2. Evolution of a CMV DNA vaccine

The first report in the literature detailing the use of DNA vaccination for CMV involved the immunisation of mice with plasmid DNA encoding the tegument protein pp65 of HCMV [24]. In this study, BALB/c mice were immunised i.m. with two plasmid constructs in which pp65 expression was controlled by either the human β-actin promoter or the HCMV immediate-early (IE) promoter. After 100 μg booster given 5 weeks post initial immunisation, approximately 60% of mice exhibited antibody responses to pp65. Antibody titres were found to be higher in mice immunised with pp65 under the control of the HCMV IE promoter, which was thought to be due to higher levels of expression. This study generated considerable interest in the field of CMV vaccinology and provided a basis for future investigations into the use of plasmid DNA constructs as mediators of immune responses against CMV. What this study did not present was any data on the ability of this pp65 DNA vaccine to induce specific cell-mediated immunity.

3. Demonstration of the protective capacity of CMV DNA vaccines

Using the murine cytomegalovirus (MCMV) IE gene 1 (pp89), the major target for CD8 T-cells in the murine CMV model, under the control of strong enhancer/promoter sequences from the HCMV IE gene, Gonzalez Armas et al. showed that immunisation with this construct could confer protection against MCMV infection [25]. This DNA vaccine elicited the production of pp89-specific CTLs which afforded a 45% (mean) protection against lethal challenge and highly significant reductions in spleen and salivary gland viral titres relative to control immunisations (up to 66-fold). A subsequent DNA vaccination study from the same group identified a new viral gene product, M84, a non-structural protein with amino acid homology to HCMV pp65 which confers protection against viral replication in BALB/c mice spleens [26,27].

4. CMV DNA vaccines that stimulate both arms of the immune system

The generation of both humoral and cell mediated immunity are believed to be necessary requisites for an effective CMV vaccine [7]. Endresz et al. used a 'cocktail' DNA vaccine approach comprising plasmids encoding two HCMV (Towne strain) proteins, gB and pp65 to generate gB-specific neutralizing antibody and pp65-specific CTL responses in BALB/c mice [28]. Two gB DNA vaccine constructs were made incorporating full-length gB and a truncated gB lacking the transmembrane domain. Mice immunised with the truncated gB exhibited higher titres of ELISA and neutralising antibodies than did those receiving full-length gB. Antibodies to full-length gB were predominantly of the IgG2a isotype, whereas those to truncated gB were mainly IgG1, suggested by the authors to be due to different antigen presentation mechanisms. All mice co-immunised with full-length gB and pp65 constructs developed gB- and pp65 specific ELISA antibodies that were shown to persist over the 31-week assay period. Of the co-immunised mice sacrificed for CTL analysis, 80% developed pp65-specific CTL responses as demonstrated in a Cr-release assay. The generation of gB-specific CTL activities was not tested in this study.

This study showed a lack of interference between the gB and pp65 constructs when co-immunised into mice at the same site and thus paved the way for future work involving a multi-target approach to DNA vaccination against CMV.

Guinea pig cytomegalovirus (GPCMV) is an excellent animal model for the development of vaccines against congenital CMV infection as it causes disease in utero. Schleiss et al. has used this model to test plasmid constructs targeting two GPCMV antigens, gB and pp65 [29]. This study showed that after epidermal immunisation of guinea pigs, all gB-immunised animals produced anti-gB antibody titres comparable to natural infection. Antibody responses to pp65 were also demonstrated in all immunised animals.

5. Enhancement of the immune response to a CMV DNA vaccine

Many groups have attempted to enhance the immune response to DNA vaccines by methods including the co-administration of various immunomodulators (cytokines, chemokines, costimulatory molecules), delivery of plasmids in liposomes and the use of experimental adjuvants [30]. The downside of these approaches with regards to prophylactic and therapeutic vaccine applications is that they are currently unlicensed for human use. It has recently been shown that negatively charged aluminium salts, currently licensed for use in humans, can be employed to greatly enhance antibody responses to DNA vaccine-encoded genes [31]. We have used a gel formulation of aluminium phosphate to boost IgG responses to an Ad169 HCMV gB DNA vaccine. Mice given a single boost of gB plasmid at week 5 post initial immunisation attained a geometric mean IgG titre (de-

termined by immunofluorescence) of 1/5120 at week 6 (compared with 1/640 in mice given plasmid without adjuvant). Mice given two boosts (weeks 5, 10) had a geometric mean IgG titre of 1/17 800 at week 11 (1/8900 without adjuvant). Immunofluorescence has been used previously in a gB DNA vaccine study in BALB/c mice to determine IgM and IgG titres [32]. Mice were immunised with 100 μg plasmid with two further boosters given at weeks 2 and 4, respectively. Geometric mean IgM antibody titre at its peak level (1–2 weeks after second booster) was shown to be 1/54, and IgG titre at its peak level (3 weeks after second booster) was shown to be 1/262. This vaccine also induced a neutralising antibody response with a percentage reduction of input infectivity (in 1:100 diluted sera) of 74.5% in mice after the two boosts.

As regards humoral responses, the potency of DNA vaccines is often inferior to that of a protein-based vaccine based around homologous antigens. Our ongoing studies in CMV indicate that the HCMV-gB/aluminium phosphate formulation could represent an effective humoral immunity-inducing component of a multi-target DNA vaccine against this virus.

The consecutive use of a DNA vaccine priming agent and an attenuated viral vector or recombinant protein boost, involving similar heterologous antigens has proved effective for the generation of high levels of humoral and cell mediated immunity [33–35]. Attenuated recombinant viral vectors have been employed previously as vaccine candidates against HCMV either alone or as priming agents. Berencsi et al. demonstrated the induction of a murine CTL response specific for HCMV gB by adenovirus and vaccinia virus recombinants expressing gB [13]. Adler et al. have used a canarypox vector expressing gB to prime for antibody responses to a live attenuated CMV vaccine [36]. A recent study has investigated the possibility of using a gB DNA vaccine construct to prime humoral responses to a gB subunit preparation in BALB/c mice [37]. Priming with a construct encoding the secreted form of gB followed by a gB subunit boost resulted in the generation of high-titre antibody responses similar in type to those obtained previously by this group with a canarypox-gB prime/gB subunit boost. Levels of IgG2a antibodies, induced at low levels by the secreted gB DNA vaccine alone, were significantly increased by boosting with the gB subunit suggesting the activation of Th1 responses.

In conclusion, research into the development and evaluation of candidate DNA vaccines against CMV is gradually gaining momentum and with the publication of the Institute of Medicine report it is hoped that some of these vaccines will shortly be investigated in human Phase I clinical trials.

References

[1] Stagno S. Cytomegalovirus. In: Remington JS, Klein JO, editors. Infectious diseases of the fetus and newborn infant. Philadelphia: WB Saunders, 1990:240–81.

[2] Griffiths PD, Baboonian C. A prospective study of primary cytomegalovirus infection during pregnancy: final report. Br J Obstet Gynaecol 1984;91(4):307–15.

[3] Stagno S, Pass RF, Cloud G, et al. Primary cytomegalovirus infection in pregnancy. Incidence, transmission to fetus, and clinical outcome. J Am Med Assoc 1986;256(14):1904–8.

[4] Stratton KR, Durch JS, Lawrence RS. (Eds). Vaccines for the 21st century: a tool for decisionmaking. Washington DC, USA: National Academy Press, 2001.

[5] Griffiths PD, McLean A, Emery VC. Encouraging prospects for immunisation against primary cytomegalovirus infection. Vaccine 2001;19(11–12):1356–62.

[6] Prichard MN, Penfold ME, Duke GM, Spaete RR, Kemble GW. A review of genetic differences between limited and extensively passaged human cytomegalovirus strains. Rev Med Virol 2001;11(3):191–200.

[7] Plotkin SA. Vaccination against cytomegalovirus, the changeling demon. Pediatr Infect Dis J 1999;18(4):313–25.

[8] Utz U, Britt W, Vugler L, Mach M. Identification of a neutralizing epitope on glycoprotein gp58 of human cytomegalovirus. J Virol 1989;63(5):1995–2001.

[9] Britt WJ, Vugler L, Butfiloski EJ, Stephens EB. Cell surface expression of human cytomegalovirus (HCMV) gp55-116 (gB): use of HCMV-recombinant vaccinia virus-infected cells in analysis of the human neutralizing antibody response. J Virol 1990;64(3):1079–85.

[10] McLaughlin-Taylor E, Pande H, Forman SJ, et al. Identification of the major late human cytomegalovirus matrix protein pp65 as a target antigen for CD8+ virus-specific cytotoxic T lymphocytes. J Med Virol 1994;43(1):103–10.

[11] Wills MR, Carmichael AJ, Mynard K, et al. The human cytotoxic T-lymphocyte (CTL) response to cytomegalovirus is dominated by structural protein pp65: frequency, specificity, and T-cell receptor usage of pp65-specific CTL. J Virol 1996;70(11):7569–79.

[12] Riddell SR, Rabin M, Geballe AP, Britt WJ, Greenberg PD. Class I MHC-restricted cytotoxic T lymphocyte recognition of cells infected with human cytomegalovirus does not require endogenous viral gene expression. J Immunol 1991;146(8):2795–804.

[13] Berencsi K, Rando RF, deTaisne C, Paoletti E, Plotkin SA, Gonczol E. Murine cytotoxic T cell response specific for human cytomegalovirus glycoprotein B (gB) induced by adenovirus and vaccinia virus recombinants expressing gB. J Gen Virol 1993;74(Pt. 11):2507–12.

[14] Berencsi K, Gyulai Z, Gonczol E, et al. A canarypox vector-expressing cytomegalovirus (CMV) phosphoprotein 65 induces long-lasting cytotoxic T cell responses in human CMV-seronegative subjects. J Infect Dis 2001;183(8):1171–9.

[15] Pass RF, Duliege AM, Boppana S, et al. A subunit cytomegalovirus vaccine based on recombinant envelope glycoprotein B and a new adjuvant. J Infect Dis 1999;180(4):970–5.

[16] Diamond DJ, York J, Sun JY, Wright CL, Forman SJ. Development of a candidate HLA A*0201 restricted peptide-based vaccine against human cytomegalovirus infection. Blood 1997;90(5):1751–67.

[17] Urban M, Klein M, Britt WJ, Hassfurther E, Mach M. Glycoprotein H of human cytomegalovirus is a major antigen for the neutralizing humoral immune response. J Gen Virol 1996;77(Pt. 7):1537–47.

[18] Spaete RR, Perot K, Scott PI, Nelson JA, Stinski MF, Pachl C. Coexpression of truncated human cytomegalovirus gH with the UL115 gene product or the truncated human fibroblast growth factor receptor results in transport of gH to the cell surface. Virology 1993;193(2):853–61.

[19] Mach M, Kropff B, Dal Monte P, Britt W. Complex formation by human cytomegalovirus glycoproteins M (gpUL100) and N (gpUL73). J Virol 2000;74(24):11 881 1 892.

[20] Monte PD, Pignatelli S, Mach M, Landini MP. The product of human cytomegalovirus UL73 is a new polymorphic structural glycoprotein (gpUL73). J Hum Virol 2001;4(1):26–34.

[21] Gyulai Z, Endresz V, Burian K, et al. Cytotoxic T lymphocyte (CTL) responses to human cytomegalovirus pp65, IE1-Exon4, gB, pp150, and pp28 in healthy individuals: reevaluation of prevalence of IE1-specific CTLs. J Infect Dis 2000;181(5):1537–46.

[22] Robinson HL, Pertmer TM. DNA vaccines for viral infections: basic studies and applications. Adv Virus Res 2000;55:1 74.

[23] Davis HL, McCluskie MJ. DNA vaccines for viral diseases. Microbes Infect 1999;1(1):7–21.

[24] Pande H, Campo K, Tanamachi B, Forman SJ, Zaia JA. Direct DNA immunization of mice with plasmid DNA encoding the tegument protein pp65 (ppUL83) of human cytomegalovirus induces high levels of circulating antibody to the encoded protein. Scand J Infect Dis Suppl 1995;99:117–20.

[25] Gonzalez Armas JC, Morello CS, Cranmer LD, Spector DH. DNA immunization confers protection against murine cytomegalovirus infection. J Virol 1996;70(11):7921–8.

[26] Morello CS, Cranmer LD, Spector DH. Suppression of murine cytomegalovirus (MCMV) replication with a DNA vaccine encoding MCMV M84 (a homolog of human cytomegalovirus pp65). J Virol 2000;74(8):3696–708.

[27] Holtappels R, Pahl-Seibert MF, Thomas D, Reddehase MJ. Enrichment of immediate-early 1 (m123/pp89) peptide-specific CD8 T cells in a pulmonary CD62L(lo) memory-effector cell pool during latent murine cytomegalovirus infection of the lungs. J Virol 2000;74(24):11 495–1 503.

[28] Endresz V, Kari L, Berencsi K, et al. Induction of human cytomegalovirus (HCMV)-glycoprotein B (gB)-specific neutralizing antibody and phosphoprotein 65 (pp65)-specific cytotoxic T lymphocyte responses by naked DNA immunization. Vaccine 1999;17(1):50–8.

[29] Schleiss MR, Bourne N, Jensen NJ, Bravo F, Bernstein DI. Immunogenicity evaluation of DNA vaccines that target guinea pig cytomegalovirus proteins glycoprotein B and UL83. Viral Immunol 2000;13(2):155–67.

[30] Gurunathan S, Klinman DM, Seder RA. DNA vaccines: immunology, application, and optimization. Annu Rev Immunol 2000;18:927–74.

[31] Ulmer JB, DeWitt CM, Chastain M, Friedman A, Donnelly JJ, McClements WL, et al. Enhancement of DNA vaccine potency using conventional aluminum adjuvants. Vaccine 1999;18(1–2):18–28.

[32] Hwang ES, Kwon KB, Park JW, Kim DJ, Park CG, Cha CY. Induction of neutralizing antibody against human cytomegalovirus (HCMV) with DNA-mediated immunization of HCMV glycoprotein B in mice. Microbiol Immunol 1999;43(3):307 10.

[33] Ramshaw IA, Ramsay AJ. The prime-boost strategy: exciting prospects for improved vaccination. Immunol Today 2000;21(4):163–5.

[34] Schneider J, Gilbert SC, Hannan CM, et al. Induction of CD8 + T cells using heterologous prime-boost immunisation strategies. Immunol Rev 1999;170:29–38.

[35] Richmond JF, Lu S, Santoro JC, et al. Studies of the neutralizing activity and avidity of anti-human immunodeficiency virus type 1 Env antibody elicited by DNA priming and protein boosting. J Virol 1998;72(11):9092–100.

[36] Adler SP, Plotkin SA, Gonczol E, et al. A canarypox vector expressing cytomegalovirus (CMV) glycoprotein B primes for antibody responses to a live attenuated CMV vaccine (Towne). J Infect Dis 1999;180(3):843–6.

[37] Endresz V, Burian K, Berencsi K, et al. Optimization of DNA immunization against human cytomegalovirus. Vaccine 2001;19(28–29):3972 80.